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## CLASSES OF ENZYMES HAVING PROBIOTIC SIGNIFICANCE

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### **Abstract**

*Strains of P. acidilactici are widely distributed in fermenting plant material including silage, cereal mashes and pickles, potato mashes, barley malt, dried leaves and hay. It is commonly added as beneficial microbe in cheese and yoghurts and known to keep a proper balance of microflora in digestive system. Pediococci cells are spherical and arranged in tetrads, however pairs are also seen in liquid cultures. Like lactic acid cocci, Pediococcus divide along two planes of symmetry. They are non-motile and non-sporulating facultative anaerobes. Normal gut ecology have anaerobic to aerobic bacterial ratio of 10:1 and P. acidilactici has the advantage of being a facultative anaerobe bacteria. The current paper describes the classes of enzymes having probiotic and industrial significance.*

**Keywords:** Enzyme, P. acidilactici, Bacteria

### **Introduction**

Pediococcus cerevisiae later renamed as P. acidilactici was the first lactic acid bacteria to be developed in the United States as a pure starter culture. A starter culture is an inoculum of beneficial microorganisms added to food products such as meat, milk and vegetables to improve food quality and

safety. Starter cultures provide rapid lactic acid development from fermentation of

sugars added to sausage resulting in a decreased pH that retards the growth of most spoilage causing microorganisms and enhances flavor and texture of the final product.

*Pediococcus* imparts desirable attributes to cheese suggesting that they may be good dairy starters if they possess the ability to utilize the particular sugar. *Pediococci* have been used in the processing and preservation of sausages, vegetables, cheeses and soy products. It also contributes to buttery/butter scotch aroma to some wines and few styles of beer.

*Pediococcus* produce pediocins, which have antibacterial activity against both spoilage and pathogenic organisms including *Listeria monocytogenes*, *Clostridium perfringens* and some *Salmonella* and *Lactobacillus* species. They have been well explored for pediocin/bacteriocin and thus were being used as probiotics in veterinary practice despite of paucity of evidence supporting their clinical impact. However, vancomycin resistant strains of *Pediococcus* were reported in septicemia patients but their virulence was not further explored thus making it difficult to distinguish between safe probiotic strain and clinical isolate.

Even if it is considered as an opportunistic pathogen, it is doubtful that it causes any disease in healthy individuals. Studies with vancomycin have shown that *P. acidilactici* has 3.5 mg/ml as MIC which is much higher



than its trough value (5-10  $\mu\text{g/mL}$ ). There are sporadic and fragmentary reports of use of *Pediococcus acidilactici* as probiotics.

Studies of in vitro probiotic attributes of this strain is promising. It possessed all the required in vitro attributes. The strain was found to be rich in different proteolytic activities. It also possessed  $\beta$ -galactosidase, antioxidant activities and also produced lactic acid and thus conferring probiotic as well as industrial significance to the strain.

Microbial extracellular enzymes are primarily involved in keeping the cells alive by providing them with the necessary amino acid pool as nutrition. Isolation of extracellular enzyme-producing microorganisms have gained immense attention due to its application in numerous biotechnological processes such as detergents, textile, dairy industries, oil processing, surfactant production and synthesis of chiral pharmaceuticals.

Supply of enzymes is one of attribute and probable mechanism of action for probiotic bacteria also and their study in probiotic bacteria is of much significance. Till date, attention has only been paid to intracellular peptidases of LAB but only a limited

number of general peptidases having broad specificity viz. aminopeptidases, dipeptidases and tripeptidases have been purified and characterized so far. Exopeptidases are less studied as compared to endopeptidases. The isolation and characterization of these enzymes are crucial for understanding their physiological and industrial role.

### **Proteases**

Proteases are group of enzymes which hydrolyze peptide bond of proteins and break them down into polypeptide or free amino acids. Protease are present in all forms of life viz. plants, animals and microorganisms and are essential for cell growth and differentiation. Microorganisms represent an excellent source of enzymes owing to their broad biochemical diversity, susceptibility to genetic manipulation, rapid growth, requirement of limited space for cultivation etc.

The vast diversity of proteases, in contrast to their specificity of action, has attracted worldwide attention in attempts to exploit their applications in food, meat, detergent



and leather industry. Besides their commercial role, proteases are vital in physiological process like blood clotting, programmed cell death, tissue differentiation and regulation of various enzymatic cascades of metabolic reactions involving the breakdown of fats, carbohydrates etc.

There is a renaissance of interest in using proteolytic enzymes as targets for developing therapeutic agents. They are degradative enzymes of central importance because they can be employed in a number of industries to create change in product taste, texture, appearance, as well as in waste recovery.

Proteases catalyze both degradative and synthetic functions. These enzymes differ in properties such as substrate specificity, active site, catalytic mechanism, pH and temperature optima, stability profile etc. Proteases are found in several microorganisms such as virus, protozoa, bacteria, yeast and fungi.

Protease genes from several bacteria, fungi and virus have also been cloned and sequenced with the prime aim of overproduction of enzyme by gene amplification, delineation of role of enzyme

in pathogenicity and alteration in enzyme properties to suit its commercial application(s). Proteins are degraded by microorganisms and they utilize the degradation products as nutrients for their growth. The degradation is initiated by endoproteases secreted by microorganisms followed further by exopeptidases at the extra-or intracellular sites.

### **Probiotic significance of proteases**

Fermented functional foods are promising due to combined benefits of health and nutrition. Though proteolytic enzymes are not very specific but a great variety of peptides are liberated during fermentation. Bioactive peptides produced in vivo or in vitro by enzymatic hydrolysis of proteins exert physiological effects. They also play important role in biological functions such as cardiovascular, nervous, gastrointestinal and immune systems when administered orally.

Production of ACE-inhibitory peptides in situ in dairy products is the most appealing approach to generate these peptides. Bioactivities of peptides released due to



action of microbial enzymes on milk proteins include hypotensive, immunomodulatory, anticancer, hypocholesterolaemic, antimicrobial, opioid and mineral-binding. Enzymatic peptide synthesis has been frequently used to synthesize peptides for pharmaceutical and nutritional purpose. Probiotics will naturally deliver bioactive peptides.

### **Lipases/Esterases**

Lipases are considered to be the third largest group based on total sales volume. Esterase (carboxylesterase, EC 3.1.1.1) and lipase (EC 3.1.1.3) are usually known as lipolytic enzymes catalyzing the cleavage and formation of ester bonds. Esterases mainly catalyze the hydrolysis of ester bonds of short-chain triglycerides or esters (10 carbon atoms). The most significant difference between esterases and lipases is in their ability to act on surface display as lipases exhibit interfacial activation whereas esterases do not. Esterases and lipases are widely present in various organisms from bacteria to higher eukaryotes.

Due to their unique properties viz. broad range of non-natural substrate specificity, high stability in organic solvents and high

enantioselectivity (for some of them), esterases are useful for a variety of industrial applications as additives in fat and oil processing, in laundry detergents, for the synthesis of fine chemicals and pharmaceuticals, in paper making and in cosmetics. Thus these enzymes have been the subject of studies due to their potential use in applied and medical biotechnology and also for the elucidation of their unknown metabolic function.

The release of free fatty acids (FFAs) during cheese ripening due to lipolytic action contributes to the flavor development. The enzymes are mostly extracellular, although intracellular and cell bound enzymes are also reported. Especially in the dairy and food processing industry they are used extensively for hydrolysis of milk fat with the purpose of flavor enhancement or acceleration of ripening process in cheese and cheese related products.

### **Probiotic significance of Esterases/Lipases**

As level of fat determines nutritional, physical, chemical and sensory characteristics of food so it leads to a growing interest in functional foods.



Production of short chain fatty acid is a probiotic attribute significance. Butyric acid has role in colorectal cancer prevention (Pouillart, 1998). They show protective action against cardiovascular diseases by reduction of serum cholesterol and triglycerides level.

### **Peptidoglycan hydrolases**

Peptidoglycan (PG) is the major component of cell wall of Gram positive bacteria and thus essential for bacterial survival as it maintains cell shape and preserves cell integrity. Peptidoglycan hydrolases (PGH) or autolysins cleave peptidoglycan (PG) strands or cross-links of the producer strain resulting in the destruction of PG mesh and thus cell lysis. Autolysis is a complex process involving different enzymes that are classified with the generic name of peptidoglycan hydrolases.

Peptidoglycan hydrolases (PGH) represent a broad group of enzymes of different origin, but similar structure. They are classified on the basis of activity of their catalytic domain viz. (i) ð-N-acetyl-glucosaminidases (hydrolyze ð-1,4-bonds between alternating N-acetylmuramic acid and N-acetylglucosamine residues of the glycan

chain, (ii)  $\beta$ -N-acetylmuramidases (hydrolyze the N-acetylmuramyl, 1,4- $\beta$ -Nacetylglucosamine bonds), (iii) N-acetylmuramoyl-L-alanine amidase or amidase (cleave the amide bond between the lactic acid side chain of MurNAc and L-Ala of the stem peptide) and iv) carboxy- and endopeptidases (cleave peptide bonds in either the peptide side chain or in the cross-bridge peptides).

### **PGH in Lactic Acid Bacteria**

As LAB are used as starter cultures in dairy fermentation, their lysis is of special interest. Lysis results in release of cytoplasmic peptidases, lipase and other enzymes.

PGH play a major role in different processes such as daughter cell separation, cell-wall turnover, autolysis, sporulation and germination, biofilm formation, resuscitation of dormant cells and autolysis in genetic transformation. These enzymes are being studied to extend their use for different applications. Some probiotic of *Lactobacillus* species were found to interact with receptors of immune system in gastrointestinal tract through PGH-dependent processes such as autolysis and/or



cell wall turnover that release muramyl-peptides or PGH fragments.

### **Probiotic attributes of PGH**

Functional role of peptidoglycan degrading enzymes is increasingly documented as these are important for a range of bacterial processes and host-microbe interactions. Most important probiotic attribute of PGH include impact on immune response, in particular down regulation of inflammatory response through induction of anti-inflammatory mechanisms mediated by immunocompetent cells and secreted cytokines.

PGH inactivation resulted in change immunomodulatory properties. These enzymes also function as cell adhesion molecule as Acm2 from probiotic *Lactobacillus plantarum* recognized mucin, extracellular component of intestinal mucosal layer.

PGHs also possess antimicrobial property and thus they antagonize pathogens. Many human and animal pathogens are Gram positive bacteria including some enterococcal strains. Infections caused by

these bacteria become unmanageable when they acquire antibiotic resistance.

Peptidoglycan hydrolases seem to be novel and efficient alternative to antibiotics. Effectiveness of some representatives was also tested and demonstrated in vitro and in vivo in animal models. *Pediococcus acidilactici* being a probiotic strain require great knowledge of these enzymes for developing strains resistant to autolysis under stress conditions and for a better understanding of contribution of released muramyl peptides as probiotic immunomodulators and antipathogenic enzymes.

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